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Abstract

The topical, fumigant and ovicidal activity of fourteen monoterpenoids and thirty-one monoterpenoid derivatives were evaluated using the house fly, *Musca domestica*. The toxicity data of acyclic, monocyclic, and tricyclic phenols, alcohols, and ketones were compared to determine structure-activity relationships involving the monoterpenoids' structural shape, type of functional group, and degree of saturation. Monoterpenoid acetate, propionate, pivalate, trichloroacetate, and trifluoroacetate derivatives were synthesized and their insecticidal activities were evaluated. The toxicities of the monoterpenoid acetate and haloacetate derivatives were compared with each other and the parent alcohols or phenols to evaluate the influence of derivatization on toxicity. Monoterpenoid ketones were more insecticidal than alcohols in the topical and ovicidal bioassays. Pivalate and acetate derivatives were more toxic than the haloacetate derivatives in both the topical and ovicidal bioassays. Thymyl trifluoroacetate was the most effective fumigant followed by menthol and fenchone. Thymol and geranyl acetate were the most insecticidal monoterpenoid and monoterpenoid derivative in the topical bioassays. Geraniol, geranyl propionate, terpineol, carvacrol and menthone were as ovicidal as the pyrethrin standard.

Disciplines

Entomology | Environmental Health | Plant Sciences | Weed Science

Comments

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Chapter 8

Structural Requirements for Monoterpenoid Activity against Insects

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The topical, fumigant and ovicidal activity of fourteen monoterpenoids and thirty-one monoterpenoid derivatives were evaluated using the house fly, *Musca domestica*. The toxicity data of acyclic, monocyclic, and bicyclic phenols, alcohols, and ketones were compared to determine structure-activity relationships involving the monoterpenoids' structural shape, type of functional group, and degree of saturation. Monoterpenoid acetate, propionate, pivalate, trichloroacetate, and trifluoroacetate derivatives were synthesized and their insecticidal activities were evaluated. The toxicities of the monoterpenoid acetate and haloacetate derivatives were compared with each other and the parent alcohols or phenols to evaluate the influence of derivatization on toxicity. Monoterpenoid ketones were more insecticidal than alcohols in the topical and ovicidal bioassays. Pivalate and acetate derivatives were more toxic than the haloacetate derivatives in both the topical and ovicidal bioassays. Thymyl trifluoroacetate was the most effective fumigant followed by menthol and fenchone. Thymol and geranyl acetate were the most insecticidal monoterpenoid and monoterpenoid derivative in the topical bioassays. Geraniol, geranyl propionate, terpineol, carvacrol and menthone were as ovicidal as the pyrethrin standard.

Plants produce secondary metabolites or allelochemicals that include a wide range of chemical compounds. The structural classes that are encompassed by the secondary compounds include terpenoids (mono, sesqui-, and diterpenoids), amines (the insecticide pellitorine), phenolic compounds (flavonoids including hydroquinones, tannins, and rotenone), alkaloids (nicotine), and nitriles (*1*). Secondary compounds that are directly utilized as insecticides are classified as botanicals. Several examples of botanicals include the contact stomach poison rotenone (extracted from *Derris elliptica*, *Derris malaccensis*, *Lonchocarpus utilis* and *Lonchocarpus urucu*), pyrethrum (found in the flowers of plants from the genus *Chrysanthemum*), and nicotine (extracted from *Nicotiana rustica* and *Nicotiana tabacum*) (*2*).

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Presently, synthetic pesticides are utilized more commonly than botanical insecticides in agriculture. Several previously used synthetic insecticides were proven problematic due to their persistence in the environment and their toxicity to non-target organisms. This is illustrated with the chlorinated hydrocarbon DDT, its metabolite DDE (*2*), and the fumigant insecticide ethylene dibromide (EDB) (*3*). In addition, several insects have become resistant to synthetic insecticides (*4*, *5*). In light of these problems, alternative classes of synthetic compounds have been developed and alternative means of pest control are being explored. One approach that may be taken is the development of [more natural,] biodegradable pesticides based on natural products that are chemically stable, yet responsive to degradation by microorganisms and to photolysis. Derivatization of degradable natural products, rendering them relatively more stable and more toxic to the target organism, may be a viable strategy toward a more biorational approach to pest control. This has been demonstrated with the natural insecticide pyrethrum and the more photostable and more toxic synthetic pyrethroids resmethrin, permethrin, cypermethrin, and deltamethrin (*2*) and in the development of the herbicide cinmethylin that is structurally based on the monoterpenoid 1,8-cineole (*6*).

Monoterpenoids characteristically are toxic to insects, safe to mammals and readily obtainable (abundant in plants and easily synthesized in the laboratory) which renders them ideal candidates in the development of more environmentally safe insecticides. Several monoterpenoids are presently used in commercial pest control products. Citronellal is a major component of insect-repellent candles. Limonene and linalool are used in flea shampoos (*7*). Linalool is also used in insecticidal sprays for house plants, while menthol is utilized as a fumigant for tracheal mites in honey bees (*8*). Although monoterpenoids have shown toxicity to insects, most monoterpenoids are less toxic to mammals. In fact several monoterpenoids are included on the generally recognized as safe (GRAS) list and they are used as artificial flavorings in foods, as fragrances in cosmetics, and as pharmaceuticals. 1,8-Cineole a component of eucalyptus oil, is found in over-the-counter formulations of nasal and bronchial decongestants, inhalational expectorants, and external analgesics (*9*). Essential oils have also been employed as preservatives and paint solvents (*10*).

Natural Occurrence

Terpenoids are produced by many living organisms including microorganisms, higher plants, and a few animals (*11*). In plants, essential oils containing terpenoids are synthesized and stored in globules within cells, in specialized ducts and glands, or in dead cells (*1*). At one time it was believed that terpenoids with the exception of carotenoids, sterols, prenols, gibberellins, dormin, abscisin, phytol, and prenols were metabolic waste. However, radiotracer studies have shown terpenoids to be rapidly synthesized even in young tissue (*11*). Terpenoids are believed to aid plants in chemical defense against phytophagous insects, bacteria and fungi. This chemical defense strategy is illustrated in the distribution of terpenoids within the plant and the physical structures associated with these secondary substances that adorn various plant surfaces. Synthesis and distribution of the toxin are often restricted to tissues that are the most susceptible to attack (*12*). Typically young lateral leaves contain higher concentrations of monoterpenoids than mainstem leaves. Bottom mainstem leaves often contain the lowest concentrations (*13*). Physical structures associated with secondary plant substances may include glandular hairs and leaf glands. They secrete a protective

substance that repels or is toxic to the plant's enemies (*1*). The plant's age and tissue type influence its ability to synthesize and store secondary compounds. The quantity of secondary plant compounds produced is affected by climatic and edaphic conditions. Although defense against the plant's enemies is believed to be a primary role of terpenoids, it has been theorized that terpenoids may also play a part in maintaining the activity of the plant's enzyme systems during dormancy (*10*). In fact monoterpenoids are believed to aid in maintaining the respiratory coenzymes in a reduced form (*11*).

Insect herbivores often use chemical cues containing terpenoids for communication, attraction or defense. The components of these chemical signals may be sequestered dietary compounds, conversion products of the host terpenoids or manufactured *de novo* by the insect (*14*). Two species of the scentless plant bug, *Niesthrea louisianica* and *Jadera haematoloma* (Hemiptera: Rhopalidae), produce and secrete a mixture of the aromatic monoterpenoid thymol and the monoterpene hydrocarbons limonene and β-pinene from their metathoracic glands (*15*). Several monoterpenoids have been identified in the sex and aggregation pheromones of bark beetles. In *Ips typographus* verbenol is an attractant while verbenone acts as a repellent (*16*). Neral and geranial have been detected in the mandibular gland secretions of several ant species that are associated with alarm-defense and attraction-trail (*17*). *d*-Limonene, *l*-limonene, α-pinene, β-pinene, and β-myrcene were found in the poison gland secretions of *Myrmicaria natalensis* (Hymenoptera: Formicidae) (18).

Toxicity of Monoterpenoids

Most terpenes are lipophilic, depending on their state of oxidation and glycosylation. They are capable of interfering with insect herbivores' biochemical and physiological functions. Typically, plant allelochemicals are not acutely toxic to mammals or insect herbivores. A few exceptions include the terpenoids grayanotoxin and ergosterol which are toxic to mammals (*19*) and a few insecticidal plant allelochemicals including the natural pyrethrins, tobacco alkaloids, rotenoids, and steroidal alkaloids (*20*). Monoterpenoids that are acutely toxic to insects include citral, an active fumigant against house flies, *Musca domestica*, (*21*) and *d*-limonene, toxic to rice weevils, *Sitophilus oryzae*, German cockroaches, *Blattella germanica* (*22*), and *Dendroctonus* pine beetles (*23*, *24*).

Plant monoterpenoids' sublethal or chronic effects appear to be more important in the plant's defense than any acute toxic effects. The plant allelochemical's ability to repel insects and act as a feeding deterrent are the plant's first defense against polyphagous insects. The familiar yellow citronellal candle, a common repellent approach, contains citronellal a monoterpenoid from lemon grass. Cineole repels the American cockroach, *Periplaneta americana* (*25*), verbenone is a deterrent to spruce bark beetles, *Ips typographus* (*26*), and geraniol is repulsive to red flour beetles, *Tribolium castaneum* (*27*). Linalool, (+)-isopulegol, (+)-pulegol, (+)-pulegone, (-)-carvone (*28*), and *d*-limonene repel the German cockroach, *Blattella germanica* (*22*). Feeding deterrents interfere with the insect's ability to ingest and utilize food, which leads to reduced growth and prolonged development. *d*-Limonene acts as a feeding deterrent to cat fleas, *Ctenocephalides felis*, (*29*) and pulegone acts as a strong feeding deterrent to the sixth instar fall armyworm, *Spodoptera frugiperda* (*20*).

Monoterpenoids may also interfere with the developmental processes of insects beginning with embryogenesis, and continuing into molting, pupation,

metamorphosis, and adult emergence (*30*). *d*-Limonene inhibits embryonic development in the cat flea, *C. felis* (*7*) and pulegone decreases larval growth of the southern armyworm, *Spodoptera eridania*, when it is continually ingested from the fourth instar to pupation (*30*).

Plant allelochemicals may also reduce reproduction success of insects by acting as an ovipositional repellent and by reducing mating success, egg production, and egg viability. Cineole indirectly affects reproductive success of the leafhopper, *Amrasca devastans* by interfering with the sonic communication between the sexes (*31*). In addition, it is a feeding repellent and an ovipositional repellent against adult yellow fever mosquitoes, *Aedes aegypti* (*9*). *d*-Limonene inhibits egg hatch of exposed western corn rootworm eggs, *Diabrotica virgifera virgifera* LeConte (*22*).

Studies on Mode of Action

It has been demonstrated that the monoterpenoids can induce several different types of bioactivities. Modes of action for the various effects have not been determined, but there have been studies that provide some clues to their possible specific mechanisms of action.

The acute symptomology elicited in insects is typified by a remarkably fast onset of tremors, followed by rapid knockdown and eventually, death. Neurotoxic activity has been documented using electrophysiological recording techniques (*32*). The noninvasive recordings of limonene-induced neurotoxicity in the earthworm, *Eisenia foetida*, yielded a distinctive set of symptoms. The primary effects were reduced conduction velocity, spontaneous activity, rebounding of nerve potentials back up the medial giant fiber, and finally, blocking of all neural activity in the medial and lateral giant fibers. Effects were fully reversible if the concentration of the toxin was less than the lethal level. Several of the symptoms were similar to those demonstrated by dieldrin in earlier experiments using the same neurotoxicity test system (*33*). Four other monoterpenoids (pulegone, myrcene, α-terpineol and linalool) also exhibited the same neurotoxic effects in the *Eisenia fetida* assay (*34*). While that non-invasive assay did not allow determination of the exact site or mechanism of action, these studies provide circumstantial evidence that the monoterpenoids act in a manner similar to the cyclodienes. The action of the chlorinated cyclodienes and lindane is acknowledged to occur at the picrotoxinin site of the GABA (γ-amino butyric acid) receptor-ionophore complex, and as an antagonist of GABA, therefore inhibiting chloride uptake into the neuron through the chloride channel (*35*, *36*). Polyhalogenated monoterpenoids have been isolated from a marine alga, and they have been shown to elicit lindane-like GABA-antagonist effects (*37*). Acetylcholinesterase inhibition has also been reported as a possible mode of action for this class of natural products (*38*).

The repellency activity of the monoterpenoids, often species-specific and compound-specific, obviously affects the insects' sensory receptors. There are indications that coevolutionary processes have resulted in plant-insect interactions such that a monoterpenoid in a plant may be repellent to many insects, but be attractive to certain host-specific species. There are also cases in which one of the chemicals is attractive to an insect at low concentrations but repellent at higher levels. Specific studies using monoterpenoids could help elucidate their mechanisms of action as repellents.

Although monoterpenoids have definite effects on insect growth, development,

and reproduction, the specific mechanisms are not well understood. Their biosynthesis via the isoprenoid pathway is in common with juvenile hormone biosynthesis. Juvenoid activity, as characterized by an extended period in juvenile forms or additional juvenile stages, has not been observed. In contrast, there have been cases of more rapid development to the adult forms in cockroaches (*39*) and mosquitoes (unpublished). Precocenes elicit anti-juvenile hormone effects that result in precocious, unfit adult forms (*40*). The rapid-development effect does not seem to be similar to that caused by precocenes.

Research Objectives

The overall objectives of the current investigation were to evaluate the toxicity of monoterpenoids and their derivatives and to begin a systematic examination of their structural requirements for bioactivity against insects. To accomplish these goals, the following studies were carried out...

1.) The topical, fumigant, and ovicidal toxicity of fourteen monoterpenoids were evaluated using house flies, *M. domestica*. Comparisons were made between aromatic, acyclic, monocyclic, and bicyclic phenols, alcohols, and ketones to determine toxicity differences involving the monoterpenoids' skeletal structures, amount of saturation, and associated functional groups.

2.) Monoterpenoid derivatives were synthesized from their alcohols or phenols and their toxicities were evaluated with topical, fumigant, and ovicidal house fly bioassays. The toxicities of the monoterpenoid derivatives were compared with the toxicities of the parent alcohols or phenols and related derivatives to determine the influence of derivatization.

Experimental Methods

Monoterpenoids. The monoterpenoids evaluated in the house fly, *M. domestica*, topical, fumigant, and ovicidal bioassays include carvacrol, (-)carveol, geraniol, linalool, *l*-menthol, menthone, pulegone, α-terpineol, thujone, thymol, (S)-*cis* verbenol, verbenone (Aldrich Chemical Company, Milwaukee, WI), *d*-carvone, and *l*-fenchone (Pfaltz and Bauer, Waterbury, CT) (Figures 1 and 2). The purity of the monoterpenoids ranged from 85-99%. The standards for comparison included chlorpyrifos (DowElanco, Indianapolis, IN), 20% pyrethrins (Pet Chemicals, Miami Springs, FL), and dichlorvos (Chem Service Inc., West Chester, PA).

Derivative Synthesis. Monoterpenoid derivatives were synthesized by reacting the parent alcohol or phenol (1 mol) with one of the acetic anhydrides (2-3 mol of acetic anhydride, trimethylacetic anhydride, trichloroacetic anhydride, or trifluoroacetic anhydride) or an acetyl chloride (2-3 mol of propionyl chloride) in the presence of methylene chloride (30 ml) and pyridine (8-20 drops) (Figure 3) (*41*). Reaction products were purified by preparatory thin layer chromatography (254 nm fluorescent-indicator silica gel with 9:1 hexane:ethyl acetate) and/or column chromatography (silica gel and 9:1 hexane:ethyl acetate). The identity of the purified samples was confirmed by comparison of their nuclear magnetic resonance (NMR) spectra with those of the standards (*42*).

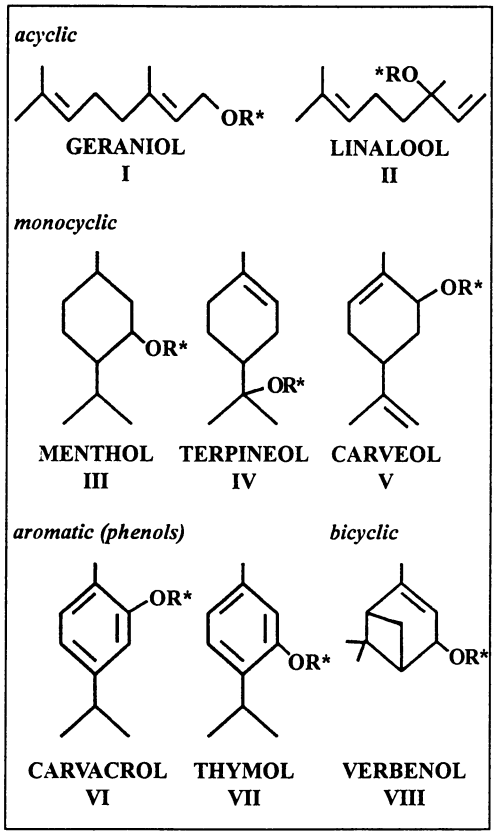

Figure 1. Monoterpenoid alcohols. *R = H

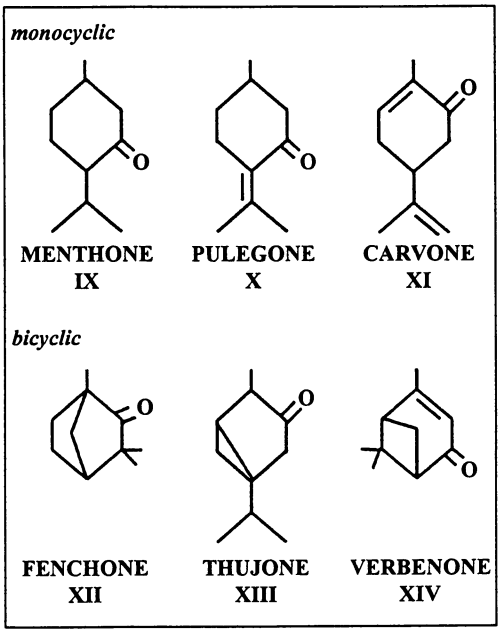

Figure 2. Monoterpenoid ketones.

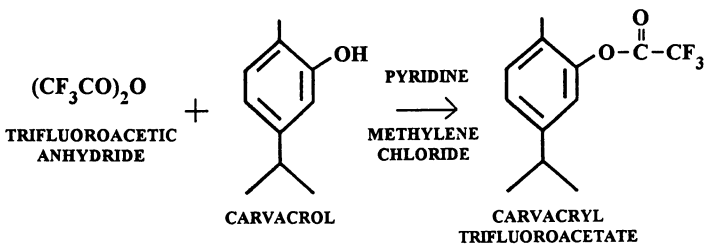

Figure 3. Typical synthesis of acyl monoterpenoid derivatives.

Biological Assays

Evaluation of Topical Toxicity. The acute topical toxicity of the monoterpenoid alcohols, ketones, and derivatives were evaluated using house flies, *M. domestica*. One microliter of the monoterpenoid or monoterpenoid derivative in acetone was applied to the pronotum of an anesthetized fly (10 days post-eclosion) with an electric microapplicator. Range-finding bioassays determined the appropriate testing concentrations. Chlorpyrifos and pyrethrins served as the standards for comparison. Acetone was utilized as the control. Each compound was evaluated at four concentrations. A minimum of three replications with ten flies per replication were obtained for each of the concentrations in the final bioassays. Mortality was assessed at 24 h. The trimmed Spearman-Karber method was used to determine the LD_{50} (*43*). Additional replications were added as necessary to narrow the confidence intervals.

Evaluation of Fumigant Toxicity. Fumigant activity of the monoterpenoids and monoterpenoid derivatives were evaluated using adult *M. domestica*. Ten house flies were placed inside a small cage that was suspended in a test chamber containing a monoterpenoid, a derivative or the dichlorvos standard (*42*). The fumigant activity of each compound was evaluated at four concentration levels. Each test was replicated at least three times. Mortality was assessed at 14 h and the LC_{50} [μg of compound/cm^3 (volume of test chamber)] was calculated using the trimmed Spearman-Karber method (*43*).

Evaluation of Ovicidal Toxicity. Approximately 50 *M. domestica* eggs (less than 12-h old) were wetted in 1500 μl of the 833 μg/ml treatment, standard (pyrethrins), or control (corn oil) solutions (*42*). The bioassays were terminated when the control eggs hatched (no less than 4 days). At least four replications were tested for each of the compounds. The total number of treated eggs and the number of hatched larvae were recorded. Percent inhibition of egg hatch was calculated using the formula described by Sharma and Saxena (*44*). Significance was determined using Chi-square analysis (*45*).

Structural Comparisons. The topical, fumigant and ovicidal toxicity data of the monoterpenoids and their derivatives were compared to determine structure-activity relationships. Acyclic aliphatic, monocyclic aliphatic, bicyclic aliphatic, and monocyclic aromatic monoterpenoids were compared to evaluate the affect of structural shape on toxicity. The influence of the type of functional groups and the degrees of saturation were evaluated in comparisons between structurally similar alcohols and ketones and structurally similar saturated and unsaturated monoterpenoids. The toxicities of structurally similar monoterpenoid acetate, propionate, pivalate, trichloroacetate, and trifluoroacetate derivatives were compared with each other and their parent alcohols or phenols to determine the effect of derivatization on toxicity (Figure 4). In the following tables, figures, and discussion, monocyclic aliphatic compounds and monocyclic aromatic compounds are referred to as monocyclic and aromatic (phenolic) compounds, respectively.

	R*
ALCOHOL/PHENOL (PARENT)	$-H$
ACETATE	$-\overset{O}{\overset{\|\|}{C}}-CH_3$
PROPIONATE	$-\overset{O}{\overset{\|\|}{C}}-CH_2CH_3$
PIVALATE	$-\overset{O}{\overset{\|\|}{C}}-C(CH_3)_3$
TRICHLOROACETATE	$-\overset{O}{\overset{\|\|}{C}}-CCl_3$
TRIFLUOROACETATE	$-\overset{O}{\overset{\|\|}{C}}-CF_3$

Figure 4. Types of acyl monoterpenoid derivatives synthesized.
*Refers to the R in Figure 1.

Results

Monoterpenoids: Aromatic vs. Acyclic vs. Monocyclic vs. Bicyclic. In the topical bioassay the phenols, carvacrol (Table I and Figure 1, compound number **VI**) and thymol (**VII**), were significantly more effective than the other monoterpenoids tested, according to the trimmed Spearman-Karber analysis. The acyclic alcohol geraniol (**I**) was more insecticidal than the monocyclic (**III, IV, V**) and the bicyclic (**VIII**) alcohols. Acyclic alcohols (**I, II**) were more ovicidal than monocyclic (**III, V**) and bicyclic (**VIII**) alcohols, with the exception of α-terpineol (**IV**). There were no apparent structure-activity relationships noted between the phenols and the acyclic, monocyclic, and bicyclic alcohols (**VI, VII, I, II, III, IV, V, VIII**), in the fumigant bioassay.

Among the monoterpenoid ketones tested, monocyclic ketones (**IX, X, XI**) were significantly more insecticidal than bicyclic ketones (**XII, XIII, XIV**) in 7 of 9 topical bioassay comparisons. Vapors of bicyclic ketones (**XII, XIII, XIV**) were significantly more active fumigants than monocyclic ketones (**IX, X, XI**) in 6 of 9 comparisons. There were no distinct trends noted involving the ovicidal activity of the monocyclic (**IX, X, XI**) and bicyclic (**XII, XIII, XIV**) ketones.

Monoterpenoids: Alcohols vs. Ketones. The topical, fumigant and ovicidal toxicity of structurally similar alcohols and ketones were compared. Ketones were significantly more toxic than alcohols (**XI** vs. **V**; **IX** vs. **III**; **XIV** vs. **VIII**) in two of three topical and ovicidal comparisons. There was no clear trend associated with the fumigant activity of the ketones and alcohols tested.

Monoterpenoids: Saturated vs. Unsaturated. The phenols carvacrol (**VI**) and thymol (**VII**) were significantly more insecticidal than the saturated (**III**), mono-unsaturated (**IV**) and di-unsaturated (**V**) monocyclic alcohols when they were topically applied to adult house flies. Menthol (**III**), the saturated monocyclic alcohol, was the most effective fumigant and one of the least effective ovicides relative to the other monocyclic alcohols evaluated (**IV, V, VI, VII**).

The monocyclic mono-unsaturated ketone pulegone (**X**) was more insecticidal than either the saturated or the and di-unsaturated monocyclic ketones (**IX, XI**) in the topical bioassays. The saturated monocyclic ketone menthone (**IX**) inhibited egg hatch more so than the structurally similar unsaturated ketones (**X, XI**). The vapors of menthone (**IX**) and pulegone (**X**) were more insecticidal to adult house flies than the monocyclic di-unsaturated ketone *d*-carvone (**XI**).

Monoterpenoids: Comparison with Standards. In the topical bioassay, the substituted phenol thymol (**VII**) was the most effective monoterpenoid (LD_{50}=33 μg/insect). However, by comparison the commercially available pyrethrin (**XVII**) and chlorpyrifos (**XV**) standards were one to three orders of magnitude more effective. The commercial fumigant, dichlorvos (**XVI**), was at least two orders of magnitude more effective than any of the monoterpenoids evaluated. However, four monoterpenoid alcohols and a single monoterpenoid ketone inhibited egg hatch as well as the pyrethrin standard (**I, II, IV, VI** and **IX** vs. **XVII**).

Monoterpenoid Derivatives: Acetates vs. Propionates. Monoterpenoid acetate derivatives were more insecticidal than their related propionate derivatives in 80% (4 of 5) of the topical bioassays (**Ia** vs. **Ib**; **IIa** vs. **IIb**; **Va** vs. **Vb**; **VIa** vs. **VIb**; **VIIa**

vs. **VIIb**) (Table II and Figure 4), and 100% of the fumigant (**IIa** vs. **IIb**; **VIa** vs. **VIb**) bioassay comparisons.

Monoterpenoid Derivatives: Acetates vs. Pivalates. The topically applied monocyclic pivalate derivatives were significantly more effective than their corresponding acetates in two of three comparisons (**IIIc** vs. **IIIa, Vc** vs. **Va, VIIc** vs. **VIIa**). In contrast the acyclic and bicyclic monoterpenoid acetate derivatives were more effective than the pivalates (**Ia** vs. **Ic, VIIIa** vs. **VIIIc**) when they were topically applied to adult house flies. There were no trends associated with the acetate and pivalate comparisons in the fumigant bioassay (**Ia** vs. **Ic**; **IIa** vs. **IIc**; **IIIa** vs. **IIIc**; **VIa** vs. **VIc**; **VIIa** vs. **VIIc**; **VIIIa** vs. **VIIIc**). Both the acetates and the pivalates were more effective fumigants than related propionate derivatives (**IIa, IIc** vs. **IIb**; **VIa, VIc** vs. **VIb**).

Monoterpenoid Derivatives: Trichloroacetates vs. Trifluoroacetates. Trifluoroacetates were more effective fumigants than the trichloroacetates (**IIe** vs. **IId**; **IIIe** vs. **IIId**; **VIe** vs. **VId**; **VIIe** vs. **VIId**). However, the acyclic trichloroacetates were more insecticidal than the acyclic trifluoroacetates (**Id** vs. **Ie**; **IId** vs. **IIe**) when they were topically administered to adult house flies.

Monoterpenoid Derivatives - Acetates vs. Trichloroacetates and Trifluoroacetates. The acetates were significantly more insecticidal than their corresponding trichloroacetates and/or trifluoroacetates in the topical and ovicidal bioassays. The exceptions to this trend were those involving thymyl trifluoroacetate and linalyl and verbenyl acetates and haloacetates in the ovicidal bioassay, and carvacryl trifluoroacetate in the topical bioassay (**Ia** vs. **Id, Ie**; **IIa** vs. **IId, IIe**; **Va** vs. **Vd**; **VIa** vs. **VId, VIe**; **VIIa** vs. **VIId**; **VIIIa** vs. **VIIIe**). In the fumigant bioassay, the vapors of the geranyl, menthyl, carvacryl and thymyl trifluoroacetates were more toxic to adult house flies than their acetates and trichloroacetates (**Ie** vs. **Id, Ia**; **IIIe** vs. **IIIa, IIId**; **VIe** vs. **VIa, VId**; **VIIe** vs. **VIIa, VIId**). In contrast, linalyl and verbenyl acetates were more effective fumigants than their related haloacetate derivatives (**IIa** vs. **IIe, IId**; **VIIIa** vs. **VIIIe**).

Monoterpenoid Derivatives: Pivalates vs. Trichloroacetates and Trifluoroacetates. Comparisons among the pivalates and the haloacetates proved to be similar to the acetate-haloacetate comparisons (see above) because the pivalates were more toxic than their related trichloroacetates and/or trifluoroacetates in the topical and ovicidal bioassays. The exception to this trend was observed in the ovicidal activity of geranyl trichloroacetate and pivalate and carvacryl pivalate and haloacetates (**Ic** vs. **Id, Ie**; **Vc** vs. **Vd**; **VIc** vs. **VId, VIe**; **VIIc** vs. **VIId, VIIe**; **VIIIc** vs. **VIIIe**). The vapors of linalyl and verbenyl pivalates were more potent fumigants than their trifluoroacetates or trichloroacetates (**IIc** vs. **IIe, IId**; **VIIIc** vs. **VIIIe**). Geranyl trifluoroacetate and menthyl trifluoroacetate were more toxic than their pivalates and/or trichloroacetates (**Ie** vs. **Ic**; **IIIe** vs. **IIIc, IIId**) in the fumigant bioassay.

Monoterpenoid Derivatives: Derivatives vs. Parent Alcohol. Several monoterpenoid derivatives gave enhanced insecticidal activity relative to their parent alcohol or phenol. (-)Carvyl acetate, propionate, and pivalate (**Va, Vb, Vc**), menthyl acetate and pivalate (**IIIa, IIIc**), geranyl acetate (**Ia**), and verbenyl acetate (**VIIIa**) are more toxic than (-)carveol (**V**), menthol (**III**), geraniol (**I**), and

verbenol (**VIII**) in the topical bioassay. The vapors of linalyl acetate (**IIa**), thymyl acetate and trifluoroacetate (**VIIa, VIIe**), and geranyl pivalate and trifluoroacetate (**Ic, Ie**) were more active fumigants than linalool (**II**), thymol (**VII**), and geraniol (**I**), respectively. (-)Carvyl pivalate (**Vc**), and verbenyl pivalate and trifluoroacetate (**VIIIc, VIIIe**) were more ovicidal than (-)carveol (**V**) or verbenol (**VIII**). Although several monoterpenoid derivatives displayed enhanced insecticidal activity, they were not as effective as the commercially available standards in the topical and fumigant bioassays. Geranyl propionate (**Ib**) inhibited egg hatch as effectively as the pyrethrins standard (**XVII**).

Discussion

Monoterpenoids are insecticidal to a variety of insects. Although their specific modes of action are unknown, acute, subacute and sublethal effects have been reported in insects and acute neurotoxic activity has been measured electrophysiologically in earthworms *Eisenia foetida* (*32*). In addition monoterpenoids may act as attractants or repellents (*22*, *28*) and they affect enzyme titers, reproduction, growth and development (*19*, *39*). The structural characteristics of monoterpenoids and their derivatives can influence their insecticidal properties. Their shape, type of functional group, degree of saturation, and type of derivatization can influence their ability to penetrate the insect cuticle, move to, and interact with the active site, and influence their degradation.

The shape, type of functional group, and degree of saturation were the structural characteristics used to evaluate structure-activity relationships of the monoterpenoids. Phenols and acyclic alcohols were more insecticidal than monocyclic and bicyclic alcohols in the topical bioassays. Similar trends were noted in the evaluation of monoterpenoids' nematicidal activity (*46*).

Ketones proved to be more insecticidal than structurally similar alcohols as an ovicide and when they were topically applied to adult house flies. This may be related to their differential susceptibility to metabolism since applications of the mixed-function oxidase inhibitor, piperonyl butoxide, resulted in greater toxicity of alkenes and alcohols (*22*). The ketone/monocyclic alcohol toxicity differences appear to play a role in the defense strategy of the peppermint plant, *Mentha piperita* (L.), against the variegated cutworm, *Peridroma saucia* (Hubner). The more toxic monoterpenoid ketones, pulegone and menthone, predominate in the younger more vulnerable leaves while the less toxic monoterpenoid alcohols and aldehydes appear in the more mature leaves (*47*).

The degree of saturation appears to influence the toxicity of the monoterpenoids considerably. The monoterpenoid phenols were topically more insecticidal than the more saturated alcohols. In the fumigant bioassay the saturated alcohol and saturated monocyclic ketone were more toxic than less saturated alcohols and ketones, respectively. In addition to the degree of saturation, the monoterpenoids' volatility is a major factor involving their fumigant toxicity in that the more volatile monoterpenoids were the more effective fumigants.

Derivatization of the monoterpenoids and evaluation of their toxicity revealed several informative trends. Acetates were more toxic than propionates in the topical and fumigant bioassays and they were more toxic than haloacetates in the topical and ovicidal bioassays. Trifluoroacetates were more effective fumigants than the acetates. This contradicted our expectations of greater toxicity with unnatural haloacetates relative to more natural acetates. The acetates' greater

Table I. Topical, fumigant, and ovicidal activity of monoterpenoids to the house fly, *Musca domestica,* adults and eggs

Compound		Insecticidal activity				
		Topical		Fumigant		Ovicidal
		LD_{50} (95% CI)[a] (μg/insect)		LC_{50} (95%CI)[a] (μg/cm^3)		% inhibition of hatch[b]
monoterpenoid alcohols and phenols						
I	geraniol	103	(95 - 112)	>1780	-----	99 j,k
II	linalool	189	(178 - 200)	6.8	(6.6 - 6.9)	87 g-j
III	menthol	193	(171 - 217)	3.6	(2.5 - 5.2)	47 b-e
IV	terpineol	199	(189 - 211)	74.5	(64.2 - 86.5)	99 j,k
V	carveol	282	(250 - 318)	1122	(972 - 1290)	56 c-f
VI	carvacrol	63	(60 - 65)	27.4	(23.4 - 32.0)	99 j,k
VII	thymol	33	(30 - 36)	142	(95 - 214)	78 e-h
VIII	verbenol	229	(220 - 238)	6.3	(4.6 - 8.8)	0 a
monoterpenoid ketones						
IX	menthone	148	(136 - 162)	13.7	(12.5 - 15.0)	100 j,k
X	pulegone	78	(77 - 80)	9.2	(8.2 - 10.4)	78 e-h
XI	carvone	157	(147 - 168)	19.0	(15.5 - 23.2)	64 c-g
XII	fenchone	295	(282 - 310)	3.8	(3.6 - 3.9)	74 e-h
XIII	thujone	198	(181 - 217)	11.9	(9.5 - 14.8)	70 d-g
XIV	verbenone	176	(162 - 192)	7.7	(7.0 - 8.4)	65 c-g
standards						
XV	chlorpyrifos	0.08	(0.07-0.10)	-----	-----	-----
XVI	dichlorvos	-----	-----	0.01	(0.009 - .012)	-----
XVII	pyrethrins[c]	0.94	(0.78-1.1)	-----	-----	100 j,k

[a]95% confidence intervals (CI) were not adjusted for multiple inferences. The monoterpenoids' activity is considered significantly different when the 95% CI fail to overlap. Trimmed Spearman-Karber analysis was used to determine LD_{50}, LC_{50} and 95% CI (*43*).
[b]% inhibition of egg hatching = 100(X-Y)/X, X= control % hatch, Y = treated % hatch (*44*). Chi-square analysis was used to determine significance at 5% (*45*). Compounds that do not share a common letter are significantly different.
[c]adjusted for 20% active ingredient

Table II. Insecticidal Activity of Monoterpenoid Derivatives to the House Fly, *Musca domestica*

Monoterpenoid derivatives		Insecticidal activity				
		Topical		Fumigant		Ovicidal
		LD_{50} (95% CI)[a] (μg/insect)		LC_{50} (95% CI)[a] (μg/cm^3)		% inhibition of hatch[b]
Ia	geranyl acetate	55	(50 - 60)	>91	-----	89 b,s,u
Ib	geranyl propionate	309	(295 - 324)	-----	-----	100 a
Ic	geranyl pivalate	93	(88 - 99)	52	(42 - 65)	77 j,l,m,n,u
Id	geranyl trichloroacetate	135	(133 - 136)	-----	-----	44 e-h,k,m,o,r,t
Ie	geranyl trifluoroacetate	>500	-----	11	(3 - 38)	0 c,d
IIa	linalyl acetate	245	(234 - 256)	4.8	(4.1 - 5.7)	0 c,d
IIb	linalyl propionate	>1000	-----	66	(39 - 114)	-----
IIc	linalyl pivalate	-----	-----	10	(9 - 11)	-----
IId	linalyl trichloroacetate	333	(304 - 365)	>209	-----	0 c,d
IIe	linalyl trifluoroacetate	663	(605 - 727)	50	(44 - 58)	0 c,d
IIIa	menthyl acetate	147	(137 - 158)	43	(42 - 44)	52 e-k,m,o,p
IIIc	menthyl pivalate	85	(73 - 98)	56	(48 - 65)	23 d-f,q,r,t
IIId	menthyl trichloroacetate	------	-----	>108	-----	-----
IIIe	menthyl trifluoroacetate	-----	-----	35	(32 - 39)	-----
Va	carvyl acetate	111	(104 - 119)	-----	-----	73 j,l,m,n,p
Vb	carvyl propionate	206	(192 - 220)	-----	-----	52 e-k,m,o,p
Vc	carvyl pivalate	88	(83 - 94)	-----	-----	88 b,n,s,u
Vd	carvyl trichloroacetate	>700	-----	-----	-----	24 d-f,q,r,t
VIa	carvacryl acetate	107	(96 - 118)	48	(39 - 58)	93 b,s,u
VIb	carvacryl propionate	134	(131 - 137)	>188	-----	3 d,q,r,t
VIc	carvacryl pivalate	-----	-----	28	(25 - 31)	0 c,d
VId	carvacryl trichloroacetate	139	(128 - 151)	>114	-----	0 c,d
VIe	carvacryl trifluoroacetate	114	(100 - 131)	27	(22 - 34)	0 c,d
VIIa	thymyl acetate	94	(84 - 104)	40	(19 - 87)	57 f-k,m,o,p
VIIb	thymyl propionate	101	(81 - 127)	-----	-----	-----
VIIc	thymyl pivalate	80	(65 - 99)	90	(69 - 120)	-----
VIId	thymyl trichloroacetate	184	(166 - 204)	208	(202 - 423)	0 c,d
VIIe	thymyl trifluoroacetate	119	(111 - 129)	2.2	(1.4 - 3.4)	44 e-h,k,m,o,r,t
VIIIa	verbenyl acetate	117	(104 - 130)	32	(27 - 40)	0 c,d
VIIIc	verbenyl pivalate	213	(181 - 251)	8.9	(7.4 - 10)	71 h-j,l,m-p
VIIIe	verbenyl trifluoroacetate	>600	-----	>83	-----	93 b,s,u

[a]95% confidence intervals (CI) were not adjusted for multiple inferences. The monoterpenoids' activity is considered significantly different when the 95% CI fail to overlap. Trimmed Spearman-Karber analysis was used to determine LD_{50}, LC_{50} and 95% CI (*43*).
[b]%inhibition of egg hatching = 100(X-Y)/X, X = control % hatch, Y = treated % hatch (*44*). Chi-square analysis was used to determine significance at 5% (*45*). Compounds that do not share a common letter are significantly different.

activity in the topical and ovicidal bioassays may be explained by their greater stabilities than those of the trichloroacetates and trifluoroacetates. In fact, several of the haloacetate derivatives even degraded during cold storage (8°C). The trifluoroacetate derivatives were more potent fumigants than most of the related acetate, propionate and trichloroacetate derivatives tested. This is believed to be associated with the enhanced volatilities of the trifluoroacetate derivatives. The addition of methyl, chlorine, or fluorine substituents may influence the volatility and stability of the monoterpenoids, affect their rate of penetration into the insect cuticle, or inhibit the insect's ability to degrade the compound once it penetrates.

Conclusions

The results of the studies provide some indications that minor structural variations (ketones vs. alcohols, phenols vs. acyclic aliphatic, monocyclic aliphatic and bicyclic aliphatic alcohols) can elicit major differences in the monoterpenoid's toxicity. These results are supported by previous research that noted a difference in response of insects to isomers of the same compound (*23*, *19*), and to monoterpenoids that differ in molecular configuration and position of functional groups (*28*). Clearly, the basic carbon skeleton, the type of functional group, the degrees of saturation, volatility, and type of derivatization all influence insecticidal activity of the monoterpenoids and monoterpenoid derivatives evaluated here. Thymol was the most effective monoterpenoid/monoterpenoid derivative tested in the topical bioassays while its derivative thymyl trifluoroacetate was the most effective fumigant. Geraniol, geranyl acetate, terpineol, carvacrol, and menthone were the most ovicidal monoterpenoid/monoterpenoid derivatives tested.

The data collected from this investigation was used to begin the development of a systematic examination of the structural requirements for monoterpenoid and monoterpenoid derivatives' bioactivity against insects. Synthesis of additional derivatives, together with applied spectrum-of-activity research, mode of action studies, and a more systematic quantitative structure-activity relationship (QSAR) approach are needed in order to fully understand the critical physiochemical properties that contribute to monoterpenoid and monoterpenoid derivatives' bioactivity in insects. This information would be valuable in our selection and development of analogs that could be novel biodegradable insecticides.

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